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Abstract

Crambe crambe is a Mediterranean marine sponge known to produce original natural substances belonging to two families of guanidine alkaloids, namely crambescins and crambescidins, which exhibit cytotoxic and antiviral activities. These compounds are therefore considered as potential anticancer drugs. The present study focuses on the environmental assessment of a novel *in vivo* process for the production of pure crambescin and crambescidin using sponge specimens cultured in aquarium.

The assessment was performed following the ISO 14040 standard and extended from the production of the different mass and energy flows to the system to the growth of the sponge in indoor aquarium and further periodic extraction and purification of the bioactive compounds. According to the results, the two stages that have a remarkable contribution to all impact categories are the purification of the bioactive molecules followed by the maintenance of the sponge culture in the aquarium. Among the involved activities, the production of the chemicals (particularly methanol) together with the electricity requirements (especially due to the aquarium lighting) are responsible for up to 90% of the impact in most of the assessed categories. However, the contributions of other stages to the environmental burdens, such as the collection of sponges, considerably depend on the assumptions made during the inventory stage. The simulation of alternative scenarios has led to propose improvement alternatives that may allow significant reductions ranging from 20% to 70%, mainly thanks to the reduction of electricity requirements as well as the partial reuse of methanol.

Keywords Sponge cultivation, crambescins, crambescidins, antitumor, Life Cycle Assessment, *Crambe crambe*, Life Cycle Inventory

1 Introduction

The largest portion of our planet's surface is covered by water and the biological diversity found in marine ecosystems make seas and oceans one of the most promising sources of natural resources for the future (Larsen et al., 2005; Leal et al., 2012). Among aquatic organisms, sponges are one of the most diverse invertebrates not only due to the number of species but also to the variety of morphological characters (Hooper and Van Soest, 2002). Indeed, between 7,000 and 8,000 different species have already been described, and at least twice that number is thought to exist (Hooper and Lévi, 1994; Thakur and Müller, 2004). This diversity associated to the fact that these sessile invertebrates produce a large array of secondary metabolites make sponges a good target for the search of high value added molecules (Leal et al., 2012). According to Sipkema et al. (2005a), 5,300 natural products have been isolated from marine sponges worldwide, an amount that increases annually (Blunt et al., 2013; 2012). Many of these molecules (e.g. halichondrin B, avarol, crambescidins) have shown high biological activities that make them valuable products for medical drugs development due to their anti-inflamatory, antitumor, immunosuppressive or neurosuppressive, antiviral or antibiotic properties, among others (Bergman et al., 2011b; Bondu et al., 2012; Newman and Cragg, 2004; Sipkema et al., 2005a). Eribulin mesylate is the first drug derived from a sponge natural product that entered the market in 2011 as an anticancer agent (Huyck et al., 2011).

Despite the great potential of bioactive compounds from marine origin and particularly from sponges, steady production is a key limiting factor that may hinder the development of commercial processes (Murray et al., 2013). As bioactive compounds of marine origin are present in small quantities in the producer organisms, fresh material is required in large amounts. Wild harvest only satisfies the demand partially and arises as an unsuitable production route (Bergman et al., 2011b; Osinga et al., 1999; Pomponi, 2001). Therefore, unless feasible alternatives to harvesting from the natural environment are developed, many of these target molecules will remain unexploited (Murray et al., 2013). For this reason, the current challenge is to develop efficient culture techniques for small to medium-scale production schemes (Schippers et al., 2012).

The selection of the most appropriate culture technique can depend on the nature of the target compound and its concentration within the sponge. Thus, if the organism presents a high concentration of the desired metabolite, the cultivation of adult specimens would be the best choice, while *in vitro* cell cultures may constitute a more suitable method for products found in low concentrations (Schippers et al., 2012; Sipkema et al., 2005b). However, *in vitro* cultivation systems have been found difficult to maintain in a long-term operation (Müller et al., 2004; Rinkevich, 1999).

Alternatively, aquaculture has been widely proposed as a technique to supply sponge materials, not only for the production of natural bath sponges, but more recently also for biotechnological purposes (Duckworth, 2009; Munro et al., 1999; Osinga et al., 1999; Pronzato and Manconi, 2008). Cultivation of sponges can be performed either *in situ* or *ex situ* (Bergman et al., 2011b; Louden et al., 2007; Sipkema et al., 2005b). Sea-based culture systems (*in situ* systems) consist of the construction of a sponge field where small cuttings (explants) from a parent are strung on a support for cultivation in the sea, so as to keep the organisms in their natural environment (Schippers et al., 2012). The main drawbacks of this alternative are the numerous risks to which sponges are exposed, including biological factors such as predation and fouling, but also diseases or adverse weather conditions (Schippers et al., 2012; Webster et al., 2002). These risks are turned into very fluctuant survival rates that strongly depend not only on the considered species but also on the location of the sponge field, the season and the aquaculture method (Bergman et al., 2011a, 2011b; De Caralt et al., 2010, 2007; De Voogd, 2007; Ledda et al., 2012; Louden et al., 2007; Osinga et al., 2010).

In order to circumvent these difficulties, the *ex situ* cultivation of sponges in closed or semi-enclosed systems such as aquarium has been proposed as an alternative strategy (Mohamed et al., 2008; Osinga et al., 2003). Even if this approach avoid seasonality effects and allow controlled conditions, the observed growth rates in aquarium are significantly lower than those of mariculture. The limited progress made in the cultivation of sponges under controlled conditions is due to the scarce knowledge on the optimal environmental conditions and ecological needs required by sponges to develop properly in a non-natural system (Carballo et al., 2010). In this regard, Schippers et al. (2012) suggest that *ex situ* cultivation should be performed in a semi-continuous mode instead of a batch operation, by regularly harvesting a small fraction of the culture.

This study focuses on the environmental assessment of the production of a bioactive fraction constituted by crambescins and crambescidins from the marine sponge *Crambe crambe* (Schmidt, 1862), a red encrusting sponge that is widely found in the Western Mediterranean Sea as well as in the Macaronesian archipelagos (Duran et al., 2004). Both families of guanidine alkaloids have already revealed significant cytotoxic activities, and they are considered as potential anticancer drugs (Bondu et al., 2012; Laville et al., 2009; Martín et al., 2013). The production process consists of the periodic extraction of these biocompounds keeping the organisms alive. This alternative may allow a steady and prolonged production of antitumoral compounds as a basis for a commercial application of these biomolecules.

The evaluation of the process was performed according to a Life Cycle Assessment (LCA) approach. LCA standardized methodology was used to assess the environmental impacts of the previously described novel process from a cradle to gate perspective (ISO 14040, 2006). Although other production processes involving marine organisms, such as microalgae or macroalgae, have been already addressed through a life cycle viewpoint (Aresta et al., 2005; Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; Lardon et al., 2009; Pérez-López et al., 2013), there are not available LCA studies focused specifically on the production of high value added molecules from sponges. To the best of our knowledge, this study develops for the first time a detailed life cycle inventory (LCI) and quantification of the environmental impacts associated with the production of bioactive compounds by sponges. Moreover, this paper presents a novel method to obtain the product while maintaining the organism alive. This approach prevents from the unsustainable exploitation of sponges in natural environments, where the growth of new individuals to replace those used to extract the target compounds would take such a long time that their production would be unfeasible.

2 Goal and scope definition

2.1 Objectives

The main goal of this study was to identify the environmental impacts associated with the sustainable production of two potential antitumor molecules, specifically crambescin and crambescidin, from the Mediterranean sponge *Crambe crambe*. The production process has been developed in the Institut de Chimie de Nice at the University the Nice Sophie-Antipolis (France). Furthermore, once the major hot spots (or most problematic issues) were determined, alternative scenarios were simulated and evaluated from an environmental point of view in order to suggest feasible improvement measures that reduce impacts to obtain a more sustainable process.

The study takes into account the production of the different mass and energy flows to the system, as well as the growth of the sponge in indoor aquarium and further periodic extraction and purification of the bioactive compounds. Although only crambescidins have been patented for their cytotoxic and antiviral activities (Rinehart and Jares-Erijman, 1998), recent studies suggest that also crambescins may have interesting biological properties (Bondu et al., 2012; Martín et al., 2013). Therefore, both families of guanidine alkaloids were considered as target products.

2.2 Functional unit

The functional unit is a key parameter that provides the reference to which the inputs and outputs of the product system can be related (ISO 14040, 2006). In this case, the selected functional unit was 100 mg of total bioactive fraction, including 50 mg of pure crambescin A1 and 50 mg crambescidin 816, which corresponds to the production during one year of operation for the base scenario. It should be pointed out that both products are obtained as pure compounds and they could be directly applied for pharmaceutical purposes. The total economic value of this production is estimated in roughly 7,000 €, according to a price of 70 €/mg for a similar biocompound: halichondrin B (Sipkema et al., 2005b).

2.3 Description of the system under study

The system boundaries for the assessment of the production of crambescins and crambescidins by *Crambe crambe* are shown in **Figure 1**. The stages or subsystems of the process included within the system boundaries are further described below, considering the extraction frequency and yield of the base scenario.

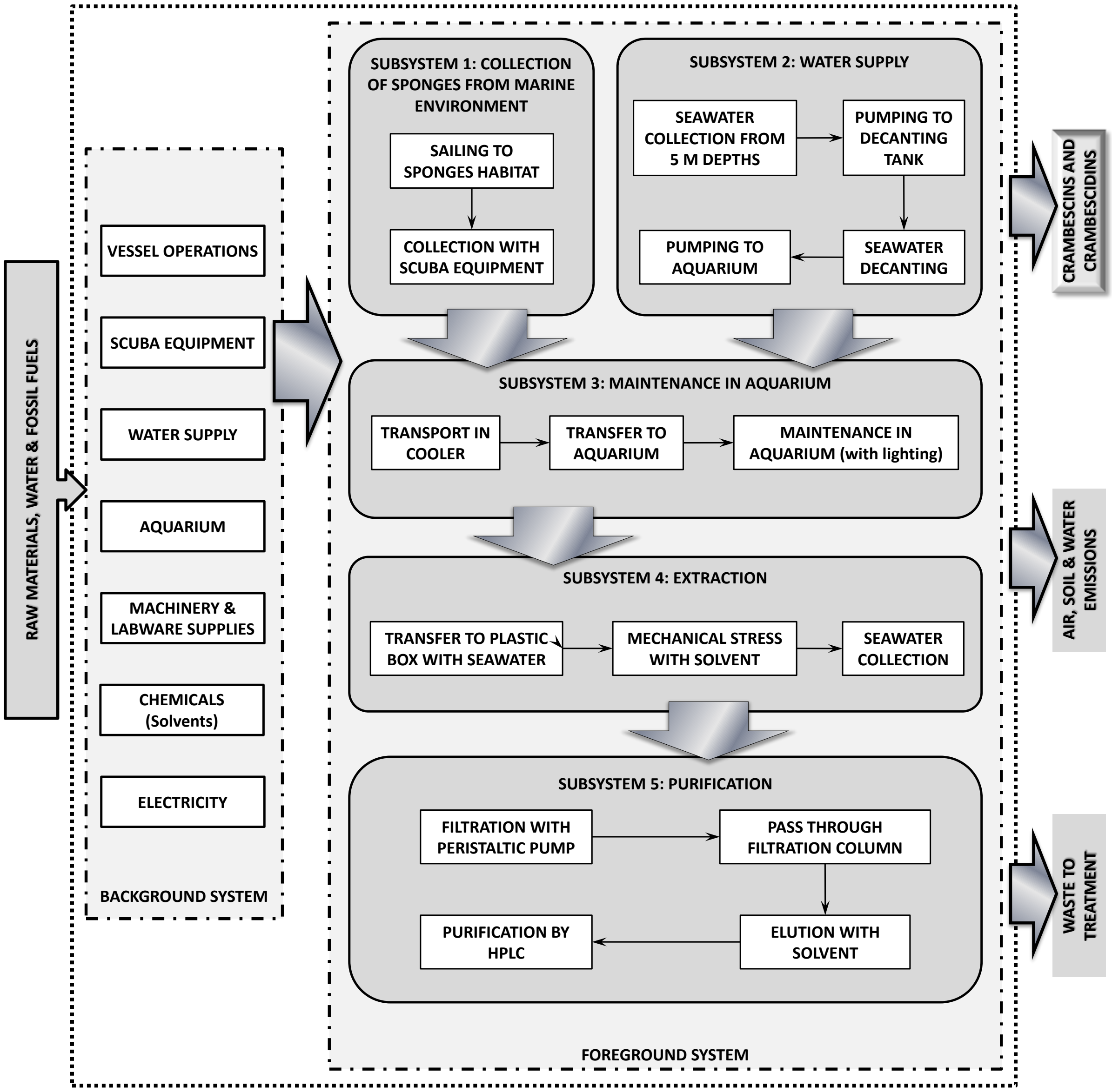

Figure 1 System boundaries and process chain of the production of pure crambescin and crambescidin from *Crambe crambe* in indoor aquariums.

i) Collection of sponges from marine environment

Specimens of the thin encrusting sponge *Crambe crambe* were collected with their substrate (hammer) at 25 m depth by scuba diving and transported in a cooler filled with seawater (18 L) in a 7 m length polyester vessel. The inventory data is based in experiments with model samples of 50 cm^2.

ii) Water supply

Three aquariums (20 L volume, 12x15x120 cm) were sustained by an open seawater circuit which pumped water from a depth of 5 m. Seawater was fed at a flow of 2.5 m^3/h and then decanted in a tank of 10 m^3, which also served as water supply for other units in the facilities. Once decanted, seawater was transferred to the aquarium at a flow rate of 2 L/min.

iii) Maintenance in aquarium

Ten individuals on their substrates, with an approximate surface of 50 cm^2 each, were transferred in each aquarium, which was illuminated by conventional fluorescent lamps. Since the aquariums were fed with seawater, operational conditions changed depending on the period of the year. The temperature was kept below 20ºC during summer with a control system, whereas it fell to 10ºC in winter. Although other parameters also fluctuated depending on the season, they exhibited values around 36.8 kg/dm^3 for salinity, 8 for pH and 6.5-7 mg/L for oxygen demand. The detailed data can be accessed on the website http://somlit.epoc.u-bordeaux1.fr/fr/spip.php?rubrique48. After two days of acclimatization in these aquariums (**Figure 2**), which could be observed by the presence of open canals and oscules on the surface of the sponge, the specimens were ready for extraction.

Figure 2 Aquariums for the indoor cultivation of *Crambe crambe*

iv) Extraction

Each individual was transferred alive into a closed plastic box (700 mL) with 475 mL seawater and 25 mL of ethanol 96%. Mechanical stress was applied using a “snail fork” and scratching with 5 cm^2 intervals, avoiding canals. Half of the volume was collected for filtration in a second closed plastic box and the stressed sponge was replaced in the aquarium for 7 days of recovering. After this period, canals and oscules were opened again in the same way as before stressing the sponge.

v) Purification of the bioactive compounds

The resulting 250 mL solution from the previous stage was filtered with a peristaltic pump through 0.22 µm filter in order to remove all insoluble particles at 50 mL/min.

After the filtration stage, the bioactive compounds were extracted from the seawater and purified by HPLC using water and methanol as solvents. The obtained fractions contained around 0.1 mg of pure crambescin A1 and 0.1 mg of crambescidin 816 (0.2 mg of bioactive compounds obtained from each individual). Both extraction and purification were repeated weekly on the same specimens. Despite the periodical scratching of the sponge surface, individuals placed in the aquarium that were not covering all the substrate were observed to grow at a rate of c.a. 20% a year (area measurement) with or without milking. No comparison was done with culture in the sea but this result evidenced that the sponges placed in aquarium were in relatively good health.

3 Inventory analysis, data quality and simplifications

The LCI data for the foreground system including chemicals, water and electricity consumptions, consisted of average data obtained by on-site measurements. Water and air emissions were calculated on the basis that the chemicals which are not consumed during the process are directly discharged. The global inventory of the process is shown in **Table 1**.

Table 1 Global inventory for the production of pure crambescin and crambescidin from *Crambe crambe* in indoor aquariums (functional unit: 100 mg bioactive fraction, consisting of 50 mg pure crambescin and 50 mg pure crambescidin).

INPUTS from TECHNOSPHERE			
Materials			
Collection of sponges from marine environment		*Maintenance in aquarium*	
Polyester (vessel hull)	0.111 kg	Polymethyl metacrylate (PMMA)	0.515 kg
Steel (engine)	0.028 kg	Lamps	0.059 kg
Anti-fouling paint	0.039 kg	*Extraction*	
Paint	0.010 kg	Ethanol	19.725 kg
Lubricant oil	0.117 kg	Distilled water	1.316 kg
Steel (scuba tank)	0.277 kg	Polypropylene	0.160 kg
Compressed air (200 bar)	8.951 kg	*Trapping and purification*	
Neoprene (scuba equipment)	0.016 kg	Steel	1.539 kg
Polypropylene (cooler)	0.016 kg	Acetonitrile	7.860 kg
Water supply		Mili-Q water	1000.0 kg
Steel	0.961 kg	Methanol	791.8 kg
Polyvinyl chloride (PVC)	2.451 kg	Trifluoroacetic acid (TFA)	1.489 kg
Concrete	51.543 kg		
Energy			
Collection of sponges from marine environment		*Maintenance in aquarium*	
Diesel	3.591 kg	Lighting	1512.00 kWh
Water supply		*Extraction*	
Pumping from sea to facilities	234.75 kWh	Filtration with peristaltic pump	2.08 kWh
Pumping from decanting tank to aquaria	144.68 kWh	Purification with flow trap column	143.20 kWh
INPUTS from ENVIRONMENT			
Materials			
Sponge biomass	159.6 g	Seawater	50875 L
Substrate	957.4 g		
OUTPUTS to TECHNOSPHERE			
Product			
Crambescins	50 mg		
Crambescidins	50 mg		
Waste treatment			
Collection of sponges from marine environment		*Maintenance in aquarium*	
Polyester	0.111 kg	PMMA	0.515 kg
Steel	0.305 kg	Polypropylene	0.559 kg
Neoprene	0.016 kg	Lamps	58.800 g
Water supply		*Extraction*	
Steel	0.961 kg	Polypropylene	0.160 kg
PVC	2.451kg	*Trapping and purification*	
Concrete	51.543 kg	Steel	1.539 kg
OUTPUTS to ENVIRONMENT			
Air emissions		**Water emissions**	
Collection of sponges from marine environment		*Collection of sponges from marine environment*	
CO_2	11.218 kg	Xylene	3.496 g
SO_2	0.007 kg	Cobalt	0.001 g
NMVOC	0.023 kg	Copper	8.122 g
CH_4	0.646 kg	Zinc	3.673 g
NO_x	0.125 kg	Ethylbenzene	0.914 g
CO	0.027 kg	Sea Nine 211	0.392 g
PM	0.013 kg	Ethanol	0.392 g
		4-methylpentan-2-one	0.392 g
		Maintenance in aquarium	
		Wastewater	50400 L
		Extraction	
		Wastewater	238.16 L
		Ethanol	9.860 kg
		Trapping and purification	
		Wastewater	1238 L
		Ethanol	9.860 kg
		Acetonitrile	7.860 kg
		Methanol	791.8 kg
		Trifluoroacetic acid	1.489 kg

Concerning the background system, the corresponding inventory data for the production of all the inputs to the system were taken from Ecoinvent database. These inputs include the production of the different chemicals required for the extraction and purification stages, the electricity used in the different production stages, as well as the materials for the equipment (vessel and scuba equipment, water supply system, aquarium, fluorescent tubes, electronic devices) and waste disposal.

In the case of the vessel, a shared use of the boat was considered for the base scenario. Thus, 1600 hours of annual operation were assumed, corresponding to 200 days of operation for 8 h/day. The amount of materials associated with the collection of sponges itself was estimated considering that this stage only requires 2 h of sailing within the whole year. The effect of this assumption will be further discussed in following sections. Emissions from fuel combustion were determined as shown in the EMEP/EEA air pollutant emission inventory guidebook of 2009 (EMEP/EEA, 2009). Chemicals related to vessel operations (i.e. paint, anti-fouling paint, marine lubricant oil) were inventoried according to Vázquez-Rowe et al. (2010), considering manufacturers' specifications. For paint and anti-fouling emitted to marine environment, a loss of two thirds of the total amount used was considered (Hospido and Tyedmers, 2005). Solid waste was assumed to be disposed of in sanitary or inert landfills

Regarding water supply, the design of the pumping and decanting system was estimated from mass balances. As the output from the decanting tank was shared with other aquariums, the corresponding amount of material was calculated from the ratio between the flow to *Crambe crambe* aquarium and the total flow to the decanting tank. The quantification of the polymethyl metacrylate of the aquarium was also calculated according to the dimensions of the tank and the density of the material, considering a wall thickness of 4 mm. As the inventory is associated with a hypothetical facility placed in shore, transport of equipments and chemicals was considered negligible. A detailed description of the corresponding database reports considered is shown in **Table 2**.

Table 2 Summary of data sources.

Energy	Electricity (French electricity profile)	Ecoinvent database (Dones et al. 2007)
	Diesel	Ecoinvent database (Jungbluth 2007)
Chemicals related to vessel operation	Anti-fouling	Vázquez-Rowe et al. (2010)
	Boat paint	
	Marine lubricant oil	
Solvents	Deionized water	Ecoinvent database (Althaus et al. 2007)
	Tap water	
	Methanol	
	Ethanol	Ecoinvent database (Sutter 2007)
	Acetonitrile	
	Trifluoroacetic acid†	
Air for scuba equipment	Compressed air	Ecoinvent database (Steiner and Frischnecht 2007)
Materials	Glass fibre reinforced plastic, polyester resin	Ecoinvent database (Kellenberger et al 2007)
	Concrete	
	Steel	Ecoinvent database (Classen et al 2007)
	Synthetic rubber	Ecoinvent database (Hischier 2007)
	PVC	
	Polymethyl methacrylate	
	Polypropylene	
	Lamp (60 W)	Ecoinvent database (Hischier et al. 2007)
Waste treatment	Inert landfill	Ecoinvent database (Doka 2007)
	Sanitary landfill	

†Assimilated to acetic acid

The materials needed for the lab ware, as well as for the vessel and scuba equipment, were estimated as average values from manufacturers' specifications. For the equipment, different life spans were considered, according to the assumptions that are specified in **Table 3**.

Table 3 Life spans and assumptions for materials' quantification.

Equipment	Component	Life span	Assumptions
Vessel	Hull (polyester)	30 years	Calculated material increased by 25% to account for vessel repairs and maintenance (Hospido and Tyedmers, 2005).
	Diesel engine (steel)	15 years	Average weight estimated from manufacturers. Estimated weight increased by 50% to account for vessel repairs and maintenance (Hospido and Tyedmers, 2005). Life span estimated from EMEP/EEA (2009).
	Anti-fouling	1 year	2 coats per year assumed, according to manufacturers.
	Paint	1 year	1 coat per year assumed, according to manufacturers.
Scuba	Diving cylinder (steel)	15 years	10 uses/year, with 1 use related to *C. crambe* process.
	Diving equipment (neoprene)	10 years	10 uses/year, with 1 use related to *C. crambe* process.
Water supply	Pumps for water supply to decanting tank (steel)	20 years	Designed for 10 m^3 decanting tank with 2.5 m^3/h flow rate. 14% of water pumped associated with *C. crambe* cultivation.
	Pipes for water supply to decanting tank (PVC)	20 years	
	Decanting tank (concrete)	20 years	
	Pumps for water supply to aquarium (steel)	20 years	Designed to feed three aquaria with 2 L/min flow rate each.
	Pipes for water supply to aquarium (PVC)	20 years	
Cooler	Polypropylene (PP)	20 years	According to manufacturers' specifications.
Aquarium	Polymethyl metacrylate (PMMA) tank	10 years	Weight calculated from on-site direct measurement of dimensions.
	Lights	30000 h	According to manufacturers' specifications.
Filtration and purification system	Plastic boxes, polypropylene (PP)	20 years	According to manufacturers' specifications.
	Pump (steel)	20 years	
	Separation columns	20 years	

In this study, two target pure products can be distinguished: crambescin and crambescidin. As both compounds have shown comparable activities, they would have similar market prices; accordingly, mass allocation was considered. Each biocompound corresponds to 50% of the total bioactive fraction, so the environmental burdens associated with them would be half of the total impacts. However, other fractions of crambescins and crambescidins may be obtained as by-products. Although these fractions have been neglected in the present study further research could provide additional information about their potential use. In this case, a fraction of the environmental impacts would be allocated to these by-products and, therefore, the environmental burdens for the main target compounds would decrease with respect to the results here described.

4 Life Cycle Assessment of crambescin and crambescidin production by *Crambe crambe*

The environmental profile of the described system was assessed by performing classification and characterization stages of the LCA methodology (ISO 14040, 2006). Normalization and weighting were not conducted as these optional (and, to some extent, subjective) elements were not considered to provide additional, robust information for the objectives of the study. The characterization factors reported by the Centre of Environmental Science of Leiden University (CML 2001 method) were used (Guinée et al., 2001). The impact potentials (or impact categories) evaluated according to the CML method were: abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP), terrestrial ecotoxicity (TEP) and photochemical oxidants formation (POFP). The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008).

4.1 Identification of hot spots

The characterization results associated with the environmental impacts caused by *C. crambe* process in the addressed impact categories are detailed in **Table 4**.

Table 4 Impact assessment results (characterization step) associated with the base scenario of the production of pure crambescin and crambescidin from *Crambe crambe* in indoor aquariums (FU: 100 mg bioactive fraction).

Impact category	**Unit**	**Value**
Abiotic Depletion (ADP)	kg Sb $_{eq}$	17.27
Acidification (AP)	kg $SO_{2\ eq}$	3.44
Eutrophication (EP)	kg $PO_4^{-3}{}_{eq}$	1.23
Global Warming (GWP)	kg $CO_{2\ eq}$	967.54
Ozone Layer Depletion (ODP)	g CFC-11 $_{eq}$	0.131
Human Toxicity (HTP)	kg 1,4-DB $_{eq}$	640.36
Freshwater aquatic Ecotoxicity (FEP)	kg 1,4-DB $_{eq}$	260.47
Marine aquatic Ecotoxicity (MEP)	kg 1,4-DB $_{eq}$	171.02
Terrestrial Ecotoxicity (TEP)	g 1,4-DB $_{eq}$	62.48
Photochemical Oxidants Formation (POFP)	g $C_2H_{4\ eq}$	319.45

As shown in **Figure 3**, most of the environmental impacts are dominated by the purification stage, with contributions ranging from 34.0% (for TEP) to nearly 90% (87.6% for ADP and 89.9% for ODP). The maintenance in aquarium is also a significant stage, especially in terms of toxicity potentials, which present values between 40.9% and 48.7%. Among the secondary subsystems, water supply is the only stage that has a relevant contribution related to toxicity potentials, with impacts ranging from 14.4% (MEP) to 21.7% (HTP).

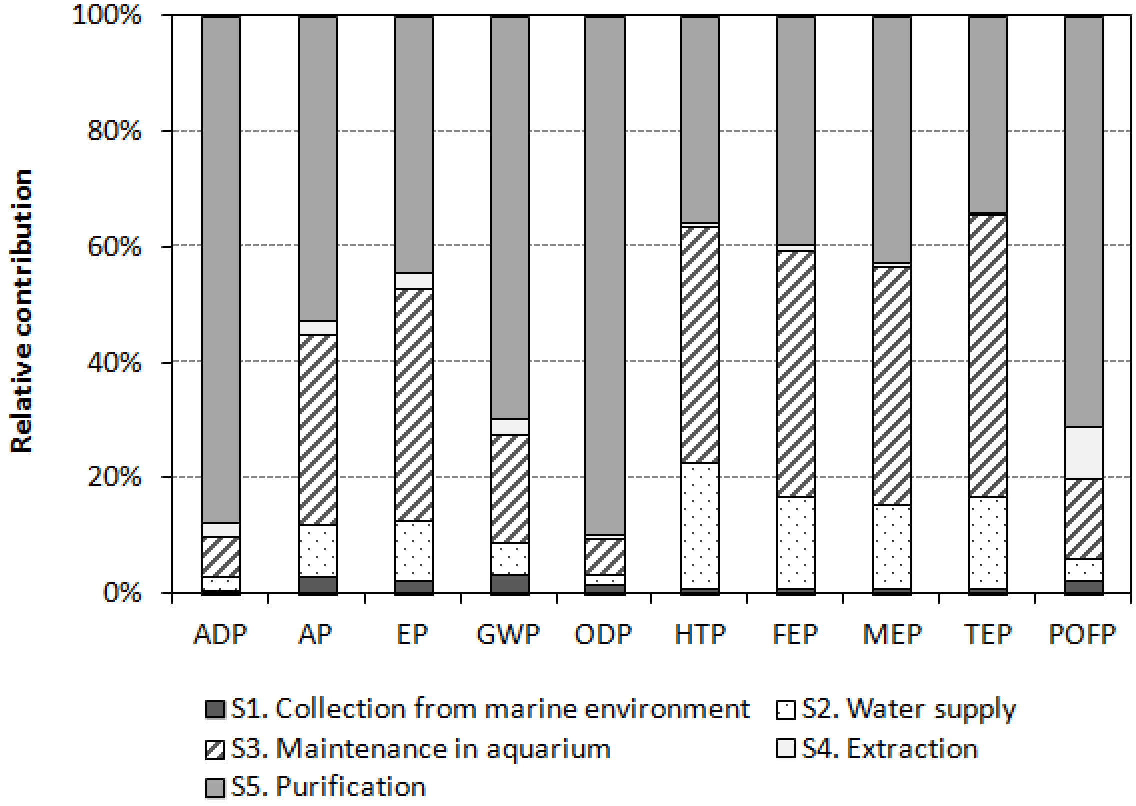

Figure 3 Relative contribution per stage of the production of pure crambescin and crambescidin in the base scenario.

Figure 4 presents the relative contribution of the different activities that are involved in the process. The production of the chemicals required in the extraction and purification stages constitutes the major impact in the categories of ADP (89.2%), AP (51.1%), GWP (70.2%), ODP (89.6%) and POFP (78.5%). Electricity is the other significant contributor to most of the environmental impacts. Indeed, this process accounts for more than 40% in six of the assessed categories, being the main cause of EP (48.7%), HTP (54.2%), FEP (53.0%), MEP (51.3%) and TEP (64.2%).

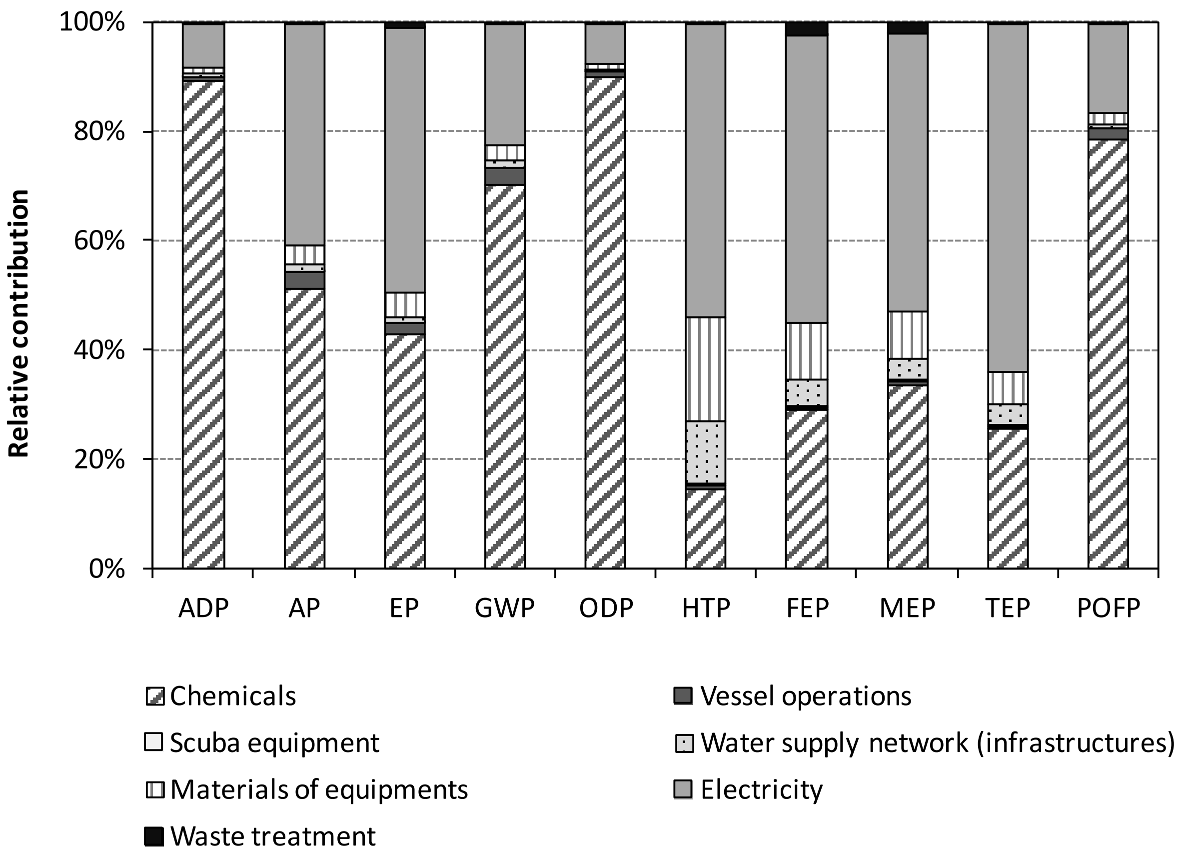

Figure 4 Relative contribution per involved activity of the production of pure crambescin and crambescidin in the base scenario.

4.2 Analysis of major contributors

4.2.1 Production of chemicals

As the production of chemicals is the main issue related to the environmental impacts of the production of crambescin and crambescidin, the breakdown of the contributions of these processes in all the assessed categories is shown in **Figure 5**. According to the graph, the production of methanol needed for the purification is the main responsible for the environmental impacts, with more than 80% of the contributions to all the categories. The reason behind this remarkable contribution is not the unitary environmental impact of methanol, but the large amount that is consumed in the process, which is two orders of magnitude above other involved chemicals with higher impacts per mass unit, such as acetonitrile or trifluoroacetic acid.

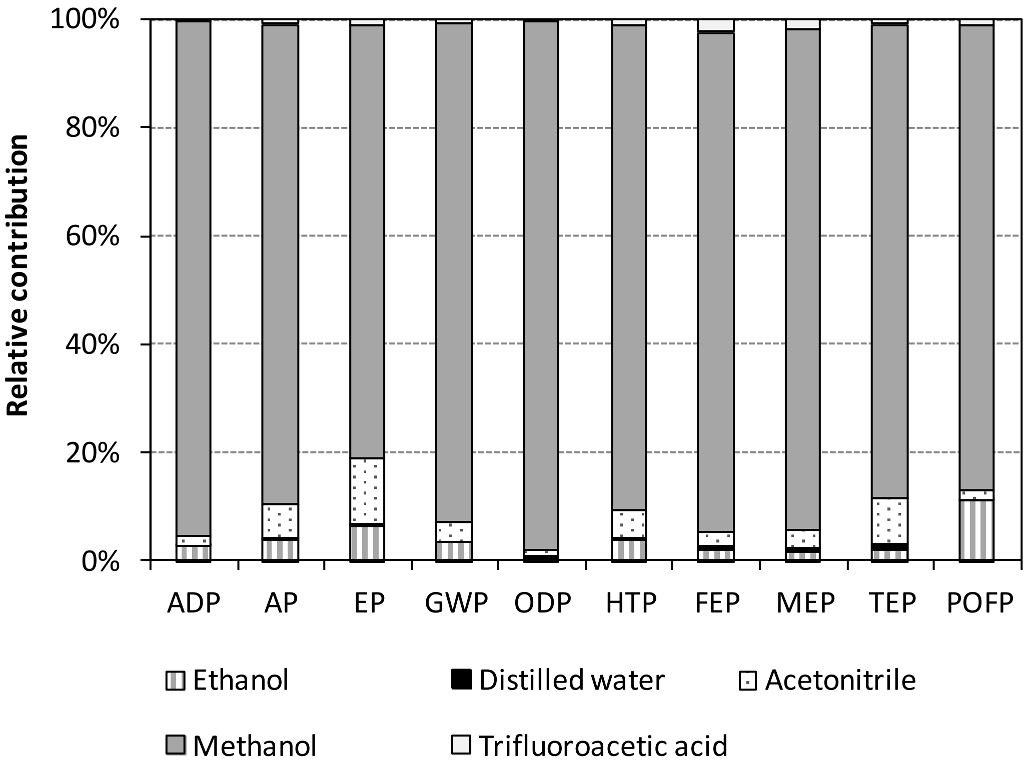

Figure 5 Relative contribution of the production of the different chemicals involved in the production of pure crambescin and crambescidin in the base scenario.

4.2.2 Electricity requirements

The second hot spot found in the analysis corresponds to the production of electricity in the different stages of the process. This process especially contributes to EP and toxicity categories. The impacts to EP are mainly due to the emissions of phosphate and nitrogen oxides whereas the contributions to toxicity categories are mostly related to the emissions of metals to air and water. Particularly, HTP is highly affected by emissions of selenium, arsenic and chromium VI, while the major responsible for FEP and MEP are emissions of nickel, vanadium and beryllium. Finally, the environmental impacts to TEP principally come from emissions of mercury derived from the use of coal for electricity generation and chromium VI from the distribution network.

In order to identify the stages with higher electricity requirements, the contributions are depicted in **Figure 6**. Nearly three fourths of the electricity consumption comes from lighting during the maintenance of *C. crambe* in the aquarium. This finding is consistent with the experience from previous works, which point out the importance of artificial lighting in the total energy cost of the cultivation of other marine organisms (Das and Obbard, 2011; Pulz and Scheibenbogen, 1998).

Therefore, the optimization in terms of electricity consumption should be focused on the reduction of lighting. Among the secondary stages, water supply has the highest consumption, with 62% due to water pumping. This result suggests that a recirculation in the seawater supply may help to reduce the environmental impacts associated with this stage.

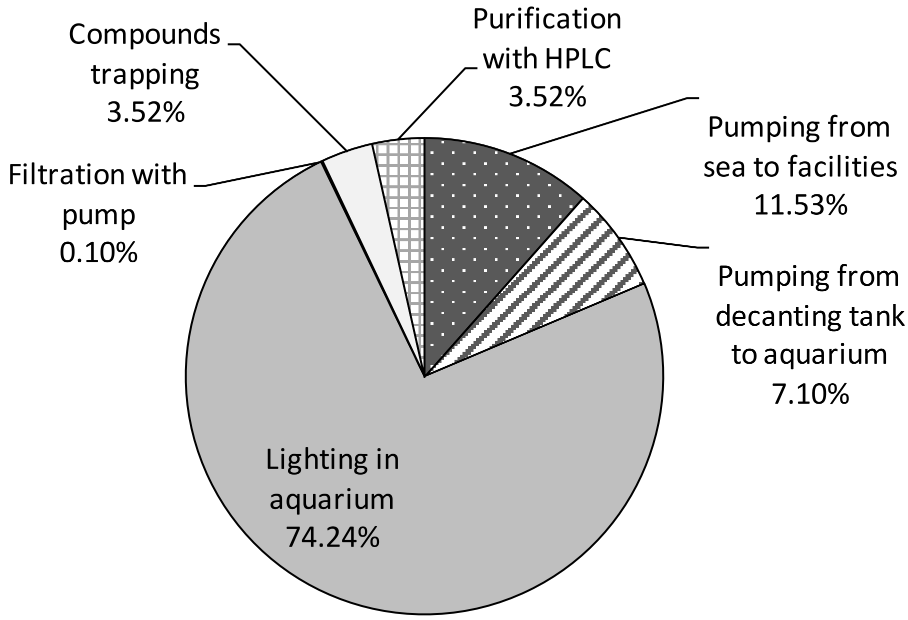

Figure 6 Relative contribution of the electricity requirements per stage to the total environmental impact of the production of pure crambescin and crambescidin in the base scenario.

4.2.3 Effect of vessel operations

According to the results, the collection of the sponges from the environment is a minor contributor to all impact categories. This contribution is mainly related to the vessel operations, including fuel consumption but also material inputs for vessel construction. Despite the limited effect found for this subsystem, the results are based on the assumption that the boat is also used for fishing. Thus, only a slight fraction of the total environmental impacts associated with vessel operations were allocated to *C. crambe* process.

Nevertheless, energy and material inputs in fishing vessels can affect the environmental profile significantly, not only due to fuel consumption but also derived from other materials, such as anti-fouling agents or paints (Hospido and Tyedmers, 2005; Vázquez-Rowe et al., 2010). Moreover, the assumptions considered to allocate the impacts from the vessel may considerably affect the global results. For this reason, a sensitivity analysis is shown in **Figure 7**.

Three alternative situations were compared to the base scenario. In the first of them (**Sc 1**), all the impacts associated with the vessel operations were allocated to the production process of crambescins and crambescidins, assuming that the boat used for the collection was a recreational vessel with no additional function (in terms of other material products obtained). However, it may be argued that a recreational use is associated with an immaterial function that should be taken into account. For this reason, the second scenario (**Sc 2**) allocates the impact of the collection of sponges according to the ratio between the number of hours associated with this process (2 h per year) and the total number of sailed hours within the year, assuming 2 h sailed per week with 52 weeks per year. The third scenario (**Sc 3**) is based on the findings of previous works, which suggest that the inputs to vessel construction and maintenance have limited contributions to the total impacts of seafood products (Hospido and Tyedmers, 2005). In this case, a fishing vessel is again considered, and building materials are excluded from the system boundaries.

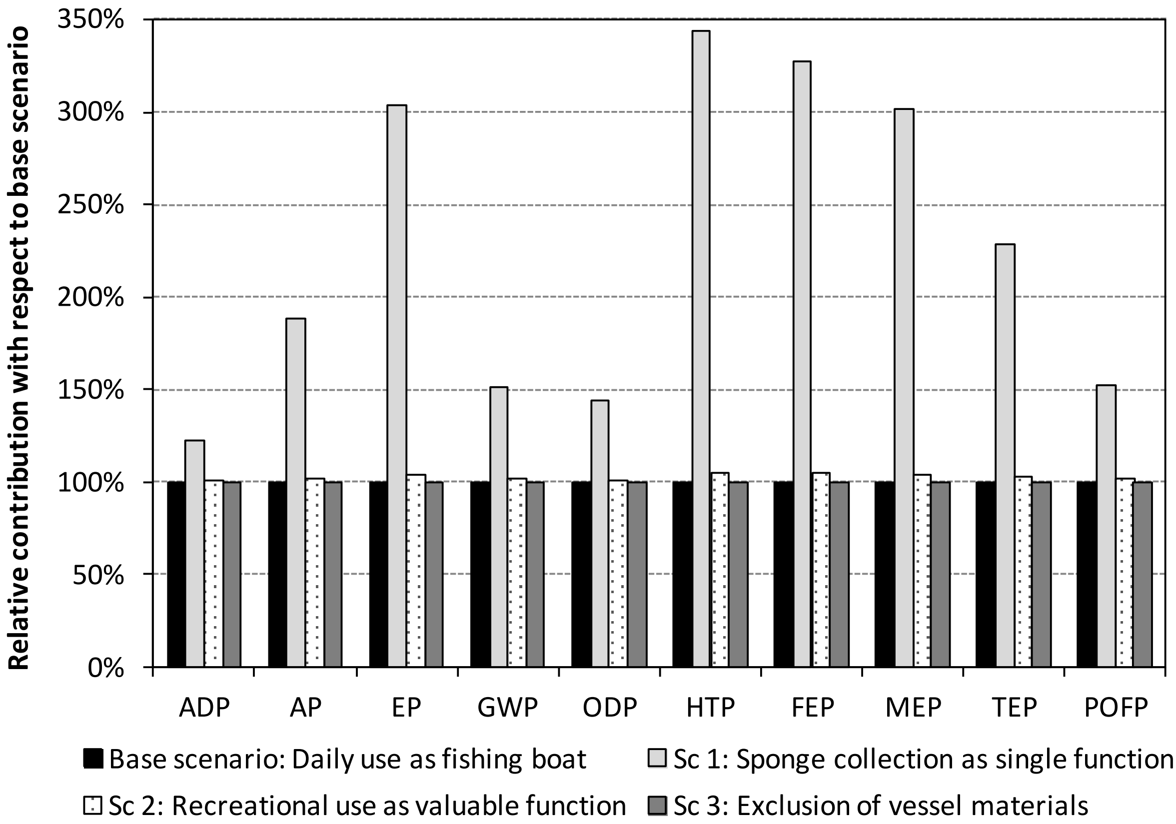

Figure 7 Effect of vessel operations in the environmental profile of the production of pure crambescin and crambescidin.

Figure 7 shows that the assumptions considered to determine the impacts from the vessel considerably influence the global environmental profile of the production of crambescins and crambescidins. Indeed, when considering that the vessel use is only associated with sponge collection (**Sc 1**), the contributions to most impact categories vary between 1.5 and 3.5 times those of the base scenario. Despite these remarkable differences, it should be pointed out that this is the most unlikely scenario, as the collection stage requires the vessel use for a very short period of time. Therefore, a combined use of the boat for other purposes, such as fishing or collection of other marine specimens for product exploitation, is expected. Regarding the other analyzed scenarios, the effect of vessel operations seems rather restricted, with deviations lower than 4.5% in all the impact categories. Thus, **Sc 2** results in impact increases between 0.4% (ADP) and 4.4% (HTP), whereas reductions observed in **Sc 3** range from 0.03% (ADP) to 0.3% (HTP) with respect to the base scenario.

5 Simulation of improvement scenarios

5.1 Solvent reuse

The production of the chemicals required for the purification stage was the principal contributor to the environmental impacts in five categories (ADP, AP, GWP, ODP and POFP). More than 85% of the mentioned contributions were specifically derived from the production of methanol, due to the large use of this solvent for the purification. For this reason, an alternative scenario is suggested, consisting of the reuse of 50% of the methanol required for obtaining of pure crambescin and crambescidin. Although this assumption was not based on experimental work, several authors have already checked the feasibility of reusing methanol to extract other similar alkaloids (Blaicher et al., 1981; Harkrader and Jones, 1998).

According to **Figure 8**, the reuse of methanol constitutes a promising option to improve the environmental profile of the studied process. The evaluated scenario presents remarkable reductions in terms of ADP (42.4%), AP (22.6%), GWP (32.4%), ODP (43.8%) and POFP (33.7%). The effect on other categories, such as HTP (6.5%) and TEP (11.0%) is relatively limited, though the performance in all the considered categories is better than the base scenario.

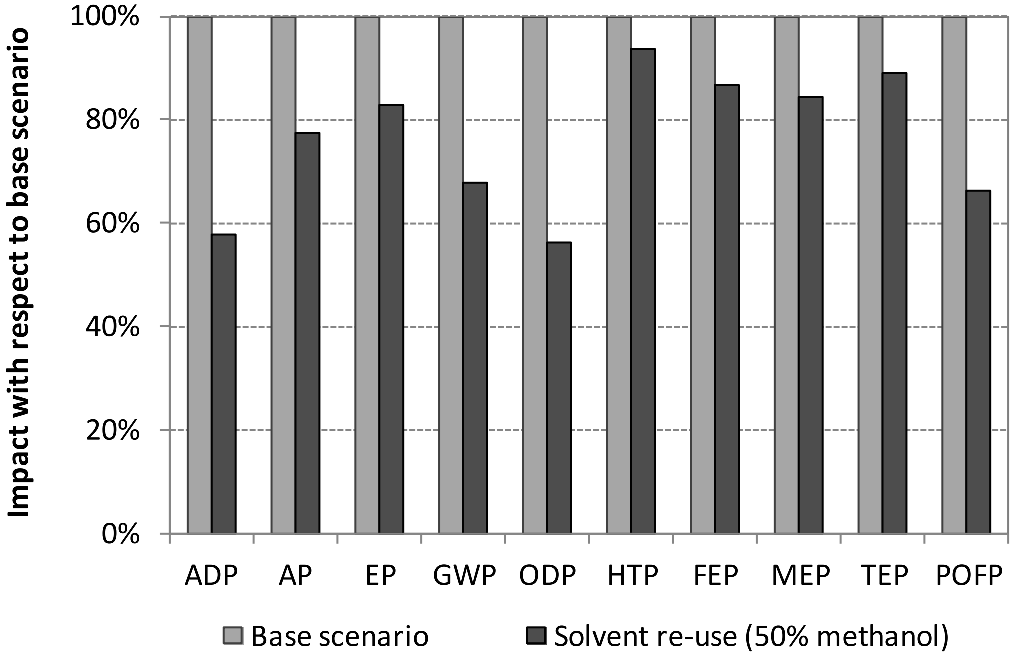

Figure 8 Effect of methanol reuse in the environmental performance of the production of pure crambescin and crambescidin.

5.2 Electricity optimization

The production of electricity required throughout the whole *C. crambe* process was identified as a major concern in six of the ten impact categories under assessment (AP, EP, HTP, FEP, MEP and TEP). The main reason is the dependence on non-renewable sources due to the use of electricity that is directly taken from the French grid, which is characterized by a limited need of fossil fuels but a high reliance on nuclear energy (Dones et al., 2007). Hence, two possible scenarios are evaluated, concerning the use of solar and wind energy as alternative sources to the electricity taken from the grid.

In addition, the artificial lighting of the aquarium was identified as the main hot spot associated with electricity requirements, with 75% of the total electric consumption. This is due to the use of fluorescent lamps, which were switched on 24 h/day. However, the necessity of light for the sponge growth is lower than for other marine organisms such as microalgae or macroalgae (González-Rivero et al., 2012; Ogbonna and Tanaka, 2000; Yeh et al., 2010). Furthermore, in the evaluated process, the main goal is not maximizing the biomass production but maintaining the sponge in such healthy conditions that allow the periodical extraction of compounds from the specimens. Therefore, an alternative regime with less lighting seems a feasible strategy to reduce the total electricity consumption of the system. Thus, two additional improvement options were proposed: 16:8 regime scenario and scenario with no lighting. Furthermore, an additional scenario was evaluated, regarding the substitution of conventional fluorescent tubes by light-emitting diodes (LEDs). As well as having a longer life span (about three times higher) than fluorescent lamps, LEDs are also more efficient and can result in a 50% decrease in energy consumption (Chen et al., 2011).

According to **Figure 9**, all the proposed alternatives show remarkable reductions in the environmental impact for most categories, except from the solar scenario in ODP which had a higher contribution mainly due to the production of materials for the solar panels. As expected, the improvements were especially significant for toxicity categories, which were more affected by the electricity requirements, but also for AP and EP. In the solar scenario, the reductions ranged from 2.5% (ADP) and 3.1% (POFP) up to 15% for HTP, FEP and MEP, and even 45.7% for TEP. The wind scenario showed the largest reductions, with more than 35% of improvement in six of the categories (AP, EP, HTP, FEP, MEP and TEP). The scenario with no lighting had the second best performance, with reductions between 30% and 50% for the same categories. However, it should be highlighted that this scenario is based on the assumption that sponge can be maintained in the same conditions (comparable growth rate and equivalent amount of bioactive compounds obtained by extraction) as in the base scenario without lighting. As the verification of this assumption would require further research, the 16:8 regime scenario seems a more feasible strategy to be applied in the short-term. Despite the more restricted improvement, the 16:8 regime scenario still showed significant reductions, ranging from 10% to 16% for those categories that are affected by the use of electricity within the process. Finally, the substitution of conventional fluorescent tubes by LEDs allowed reductions between 3.5% and 24.5%.

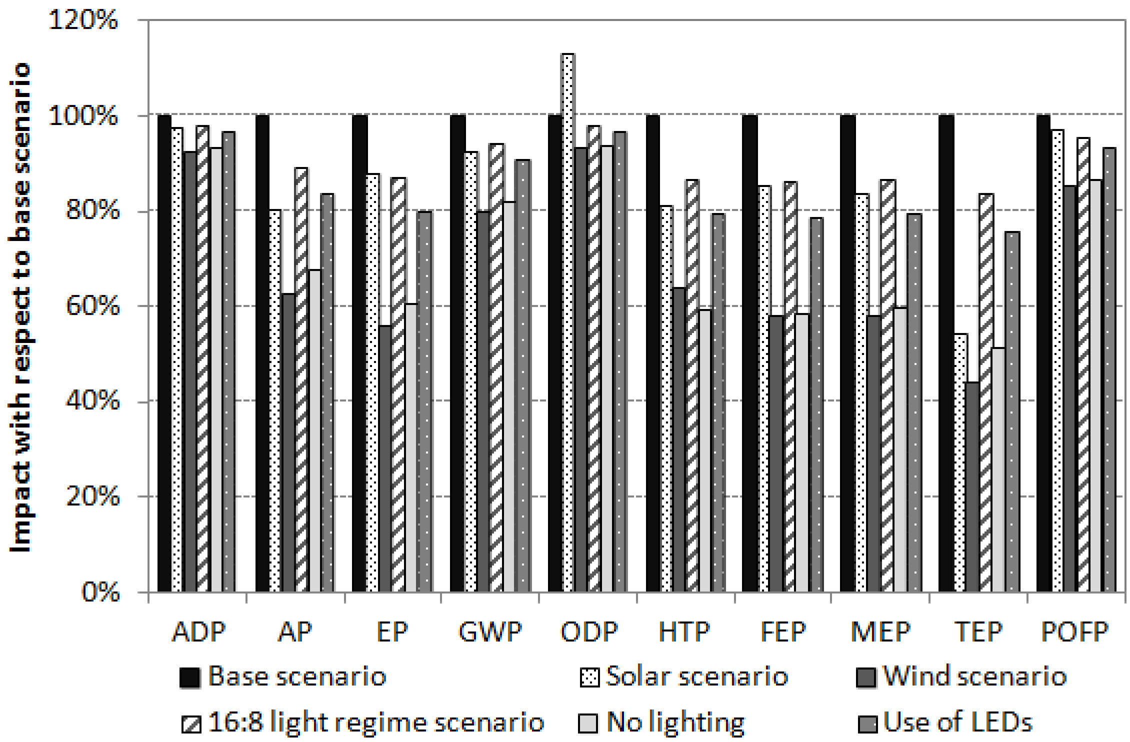

Figure 9 Sensitivity analysis of the environmental performance considering four improved alternatives for the reduction of electricity requirements in the production of pure crambescin and crambescidin.

5.3 Recirculation effect

As indicated in the previous section, water supply constitutes a secondary contributor that may have a relevant effect in some categories, due to the electricity consumption of the pumping system. Indeed, continuous pumping of water has already been identified as a significant issue in the cultivation of other marine organisms such as microalgae (Lam and Lee, 2012; Xu et al., 2011).

In the case of *C. crambe* process, this contribution is mostly associated with the seawater collection and pumping from the sea to the decanting tank. Therefore, an alternative scenario where 50% seawater was recycled to the aquarium instead of its direct discharge to the sea was assessed. However, the results indicate that the improvement achieved with this measure would be rather limited, with reductions of impact between 0.7% and 4.3%. The highest reductions are found in the toxicity categories, which were those with a significant contribution of electricity. The improvements related to these categories would range from 3.5% for HTP to 4.3% for TEP.

5.4 Improved waste treatment

Although the environmental burdens associated with waste treatment are rather slight in comparison with other subsystems of the process, an alternative option was proposed, regarding the final disposal of the waste. In the present study, the assumption that the materials of the equipment and infrastructure were finally sent to landfill was considered. Nevertheless, previous LCA studies of related processes proposed other approaches, such as sending these materials to recycling (Collet et al., 2011). In this case, the final disposal of steel, plastic materials and concrete to landfill was substituted by the recycling of these materials. However, the improvement observed with this measure is very limited and the highest reductions were between 1% and 2.4% for the categories of EP, FEP and MEP.

5.5 Best performance vs most feasible scenario

Several of the simulated scenarios can be simultaneously applied, allowing higher reductions of impact. Therefore, the compatible improvement alternatives were combined in two hypothetical scenarios:

- **Best performance scenario.** In this case, the maintenance in aquarium with no lighting is considered together with the wind electricity supply for the other electricity requirements. Additional, a methanol re-use of 50% is taken into account, along with a 50% seawater recirculation and a recycling scenario.
- **Most feasible scenario.** Despite having the lowest environmental burdens, the best performance scenario is based on the assumptions that the reduction of lighting and the re-use of the solvent neither affect the yield of the process nor the purity of the two produced compounds. As further research should be needed to prove the accuracy of these assumptions, another alternative scenario that seems more feasible in a short period of time was proposed. In this case, a 16:8 regime was considered jointly with a solar electricity supply and the use of LEDs, as well as a methanol re-use of 25%, was assumed. Seawater recirculation and recycling of materials were also considered.

The improvements that may be achieved by the combined implementation of the alternative scenarios are shown in **Figure 10**. According to these results, the environmental profile of the production of pure crambescin and crambescidin can be significantly enhanced, with reductions of impact for the best performance scenario between 52 and 78% depending on the category. Even if a more conservative approach is considered, the most feasible scenario in a short term period would allow improvements up to 40% for the categories of AP, EP, HTP, FEP and MEP, and as high as 65% for TEP.

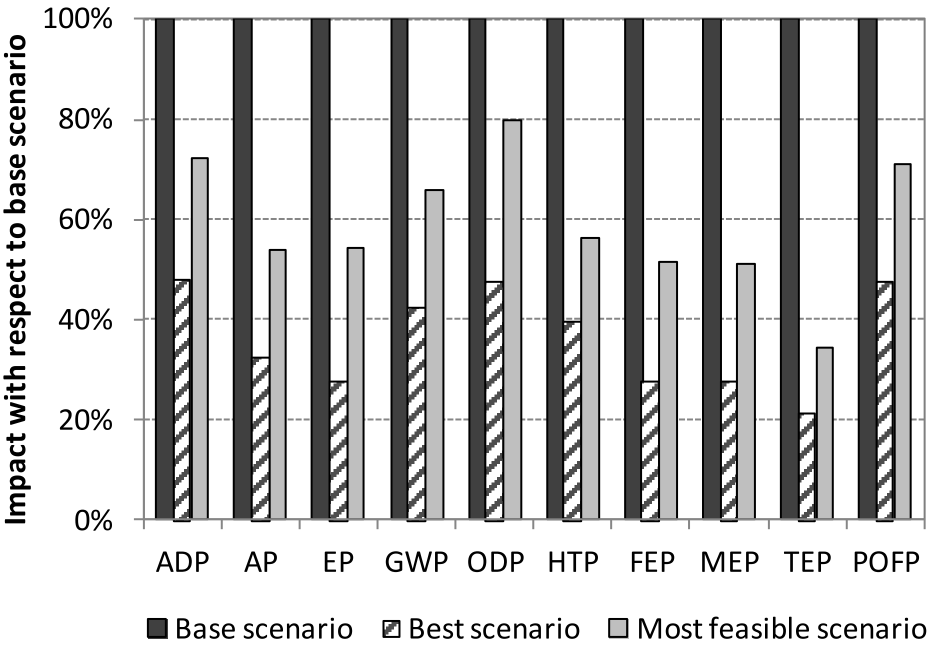

Figure 10 Comparative environmental profiles of the base scenario, the best potential scenario and the most feasible scenario.

6 Conclusions

The principal aim of this study was to evaluate the environmental performance of a novel process for the production of high value added biomolecules from a marine sponge. The life cycle approach was used to identify the main contributors to the assessed impact categories, allowing the proposal of several improvement scenarios that were simulated and analyzed through LCA methodology.

The use of large amounts of methanol during the purification stage and the high electricity requirements essentially due to the continuous illumination of the aquarium were found as the two major environmental concerns of the base scenario. The environmental burdens of other stages, such as the collection of sponges, considerably depend on the assumptions made during the inventory analysis stage.

Among the proposed improved alternatives, the most promising scenarios were those related to electricity optimization, including the reduction of electricity requirements together with the use of

LEDs and the substitution of electricity from the grid by renewable sources, as well as the partial re-use of methanol. The combined implementation of the evaluated options may allow impact reductions in the short term ranging between 20% and 65% depending on the considered category. Further environmental improvements up to 70% could be achieved according to the best potential scenario.

The results of this paper should be taken into account, not only due to the importance of LCA as a tool to develop improved production systems but also due to the novelty of the proposed process. This production process avoids the over-exploitation of the marine environment, as it replaces the need of wild harvesting by the maintenance of specimens in a closed aquarium. Moreover, the process is currently under a scale up phase and will be able to deliver enough sponge natural products to be amenable to commercialization. Therefore, this alternative may allow to overcome existing bottlenecks regarding the marine-based biotechnology and specifically in the field of sponges cultivation.

Acknowledgements This project has been developed within the framework of the BAMMBO Project (Project reference: FP7 KBBE-2010-4 - 265896) and has been partially financed by the Xunta de Galicia (Project Reference GRC 2010/37). P. Pérez-López would like to express her gratitude to the Spanish Ministry of Education for her research fellowship (AP2012-1605). We also acknowledge the assistance of D. Luquet for the recollection of sponges and the Observatoire d'Océanologie de Villefranche sur Mer (G. Gorsky) for hosting the aquariums.

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